Total mesophilic counts underestimate in many cases the contamination levels of psychrotrophic lactic acid bacteria (LAB) in chilled-stored and packaged food products

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1. Introduction

Food industries worldwide have to conform to microbial standards associated with the safety and the quality of their products. Microbiological quality standards have been established empirically by manufacturing companies however they are not applicable to all industrial processing plants and they fluctuate for numerous products of the same type. The most widely applied parameter used to determine the microbial quality of a packaged product which is stored under chilling temperature is the ISO 4833:2003 concerning the total viable mesophilic count for which incubation of the plates is performed at 30°C. At the same time psychrotrophic lactic acid bacteria (LAB) are increasingly isolated from food products deemed unfit for consumption nowadays as in populations >10^7 CFU/g they produce unpleasant odors, form slime and cause discoloration.

2. Materials and methods

A microbiological analysis was conducted for 86 various packaged (air, vacuum, modified-atmosphere) chilled-stored retail food products covering a wide range (Fig. 1), comparing the total viable counts on plates incubated at 30°C for 3 days (representing the mesophiles) and at 22°C for 5 days (indicating the psychrotrophs).

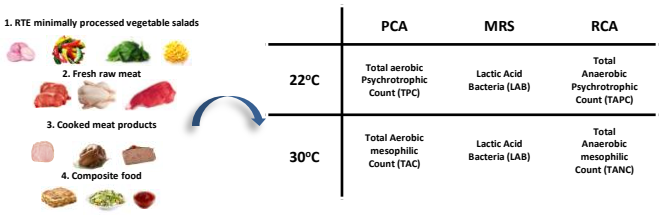

Fig 1. Microbiological analysis performed for the mesophilic flora (incubation of plates at 30°C) and the psychrotrophs (at 22°C).

Colonies were picked up from the plates incubated at 22°C when these plates were found to have higher numbers of colonies in comparison to their equivalent incubated at 30°C (>0,5 log CFU/g). rep-PCR and AFLP analysis were the two genotypic identification methods combined to facilitate the taxonomic distribution of the recovered isolates.

3. Results

In 38% of the samples (33 out of 86), the psychrotrophic counts had significantly higher values (+ 0,5-3 log CFU/g) than the corresponding total aerobic mesophilic counts (Fig. 2 & 3) and a total of 222 isolates were retrieved from the plates incubated at 22°C, 212 of which were identified as LAB.

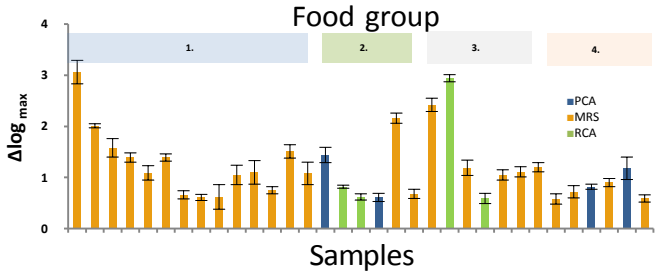

Fig 2. Presentation of the highest difference between mesophiles and psychrotrophs for each one of the 33 samples.

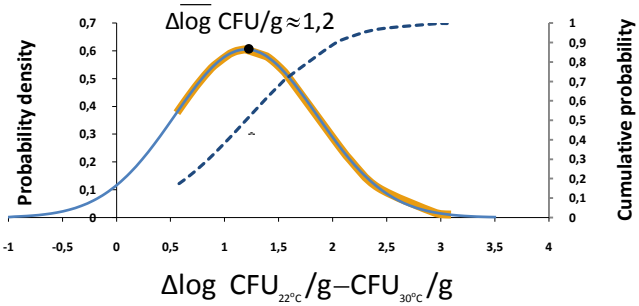

Fig 3. Presentation of the $\Delta\log_{max}$ of the 33 samples that proved to have greater counts at 22°C on a distribution curve.

The species with the highest frequency of isolation were *Leuconostoc gasicomitatum*, *Leuconostoc gelidum* and *Lactococcus piscium*-like (Table 1) being dominant in 17, 14 and 6 different samples respectively.

Table 1. Species diversity of isolated LAB and number of isolates unable to grow at 30°C after incubation for 3 days.

Species	Number of isolates	Number of isolates unable to grow at 30°C
★ *Leuconostoc gasicomitatum*	64	58
★ *Leuconostoc gelidum*	45	40
Leuconostoc carnosum	15	
Leuconostoc inhae-like	10	10
Leuconostoc mesenteroides	1	
Leuconostoc lactis	1	
★ *Lactococcus piscium*-like	27	24
Lactobacillus algidus	10	10
Lactobacillus fuchuensis	6	2
Lactobacillus sakei	3	
Lactobacillus oligofermentans	1	
Lactobacillus sanfranciscensis	1	1
Carnobacterium divergens	9	1
Enterococcus raffinosus	4	
Weissella coli	3	
Unidentified		8
Total	212	154

4. Conclusions

During the past decade many cases of food spoilage manifestations attributed to psychrotrophic LAB have been reported. *Leuconostoc* spp. are being continuously isolated from MAP or vacuum packaged products. Since 2000 the hitherto unknown species of *Leuconostoc gasicomitatum* has been involved in many cases in Northern Europe. Based on the results of the present study the shortcomings of the mesophilic enumeration technique to detect these competent bacteria could be avoided by implementing a reference shelf-life parameter focusing on the psychrotrophic flora for chilled-stored and packaged food products.

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